

**PATENT COOPERATION TREATY**  
**PCT**

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**  
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference <b>74689-A-PCT/JPW/CH</b>	<b>FOR FURTHER ACTION</b>	See item 4 below
International application No. <b>PCT/US2006/024157</b>	International filing date ( <i>day/month/year</i> ) <b>20 June 2006 (20.06.2006)</b>	Priority date ( <i>day/month/year</i> ) <b>21 June 2005 (21.06.2005)</b>
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant <b>THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK</b>		

<p>1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).</p> <p>2. This REPORT consists of a total of 10 sheets, including this cover sheet.</p> <p>In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.</p>																								
<p>3. This report contains indications relating to the following items:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%;"><input checked="" type="checkbox"/></td> <td style="width: 15%;"><b>Box No. I</b></td> <td style="width: 70%;">Basis of the report</td> </tr> <tr> <td><input type="checkbox"/></td> <td><b>Box No. II</b></td> <td>Priority</td> </tr> <tr> <td><input type="checkbox"/></td> <td><b>Box No. III</b></td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td><b>Box No. IV</b></td> <td>Lack of unity of invention</td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td><b>Box No. V</b></td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td><input type="checkbox"/></td> <td><b>Box No. VI</b></td> <td>Certain documents cited</td> </tr> <tr> <td><input type="checkbox"/></td> <td><b>Box No. VII</b></td> <td>Certain defects in the international application</td> </tr> <tr> <td><input type="checkbox"/></td> <td><b>Box No. VIII</b></td> <td>Certain observations on the international application</td> </tr> </table>	<input checked="" type="checkbox"/>	<b>Box No. I</b>	Basis of the report	<input type="checkbox"/>	<b>Box No. II</b>	Priority	<input type="checkbox"/>	<b>Box No. III</b>	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	<input checked="" type="checkbox"/>	<b>Box No. IV</b>	Lack of unity of invention	<input checked="" type="checkbox"/>	<b>Box No. V</b>	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	<input type="checkbox"/>	<b>Box No. VI</b>	Certain documents cited	<input type="checkbox"/>	<b>Box No. VII</b>	Certain defects in the international application	<input type="checkbox"/>	<b>Box No. VIII</b>	Certain observations on the international application
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<p>4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).</p>																								

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. +41 22 338 82 70</p>	<p>Date of issuance of this report <b>10 March 2009 (10.03.2009)</b></p> <p>Authorized officer <b>Masashi Honda</b> e-mail: pt08.pct@wipo.int</p>
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**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US06/24157

**Box No. I Basis of this opinion**

1. With regard to the language, this opinion has been established on the basis of:

the international application in the language in which it was filed

a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2.  This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of:

a. type of material

a sequence listing

table(s) related to the sequence listing

b. format of material

on paper

in electronic form

c. time of filing/furnishing

contained in the international application as filed.

filed together with the international application in electronic form.

furnished subsequently to this Authority for the purposes of search.

4.  In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US06/24157

**Box No. IV Lack of unity of invention**

1.  In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:  
 paid additional fees  
 paid additional fees under protest and, where applicable, the protest fee  
 paid additional fees under protest but the applicable protest fee was not paid  
 not paid additional fees
2.  This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is  
 complied with  
 not complied with for the following reasons:  
See the lack of unity section of the International Search Report (Form PCT/ISA/210)
4. Consequently, this opinion has been established in respect of the following parts of the international application:  
 all parts.  
 the parts relating to claims Nos. 1-15

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
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**Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)

Claims 1-15 YES  
Claims NONE NO

Inventive step (IS)

Claims 1-15 YES  
Claims NONE NO

Industrial applicability (IA)

Claims 1-15 YES  
Claims NONE NO

**2. Citations and explanations:**

Please See Continuation Sheet

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
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Supplemental Box  
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 1-4 and 6-15 lack an inventive step under PCT Article 33(3) as being obvious over Nyren et al (USPN 6,210,891 issued April 3, 2001) in view of Milton et al (WO 2004/018497 published Mar. 3, 2004).

Regarding claim 1, Nyren teaches a method for determining the nucleotide sequence of a single-stranded DNA comprising performing the following steps for each nucleic acid residue of the DNA whose identity is to be determined: (a) contacting the DNA under DNA polymerization-permitting conditions with (i) dNTP and (ii) (exo-) DNA polymerase (Fig. 9, Top panel, column 2, lines 24-30). Nyren et al further teaches step (b) (i) determining whether pyrophosphate is generated as a result of step (a), whereby (i) pyrophosphate generation indicates that polymerization has occurred and the identity of the nucleic acid residue in the DNA is that which is complementary to the dNTP used in part (i) of step 'a' and (2) the absence of pyrophosphate generation indicates that the identity of such nucleic acid residue is not that which is complementary to such dNTP (column 2, lines 30-38). Nyren et al also teaches that if pyrophosphate is not generated, repeating step (a) once, twice or three times as necessary, wherein in each repetition of dNTP is used which is different from any dNTP already used, and determining, after each repetition of step (a), whether pyrophosphate is generated, such generation indicating that polymerization has occurred and the identity of the nucleic acid residue in the DNA is that which is complementary to the blocked dNTP used in part (i) of the repeated step 'a' (Fig. 1, see the repeat cycle step).

Nyren et al are silent about 3'-O-blocked dNTP and 9N DNA polymerase A485L/Y409V in the pyrophosphate sequencing method. However, 3'-O-blocked dNTPs and 9N DNA polymerase (exo-) A485L/Y409V, for incorporating nucleotides were known at the time of the claimed invention was made as taught by Milton et al, who teaches dNTP comprising an allyl group bound to 3' oxygen (Abstract) that includes 3'-O-allyl dATP (Structure # 55, pg. 111, lines 1-5), 3'-O-allyl dCTP (Structure # 43, pg. 98, lines 25-30), 3'-O-allyl dGTP (Structure # 48, pg. 104, lines 21-27) and 3'-O-allyl dTTP (Structure # 37, pg. 91, lines 1-5, pg. 93, lines 5-8), thus teaching 3'-O-blocked dNTPs. Milton et al also teaches the incorporation of blocked nucleotides into polynucleotides using and 9N DNA polymerase (exo-) A485L/Y409V (Fig. 5 and pgs. 84, lines 27-29 and pg. 86, lines 28-30).

Milton et al also teaches step 'c' of removing from the 3'-O-blocked dNTP polymerized in step (a) or (b), whichever is applicable, the moiety blocking the 3'-O-atom of the dNTP, with the proviso that such removing step is optional in the event that there remains no further nucleic acid residue of the DNA whose identity is to be determined (pg. 86, lines 1-26). Milton et al also teaches that dNTP containing 3'-O-blocked group provides a method of controlling the incorporation of nucleotide molecule complementary to the nucleotide in a target single stranded DNA to determine its sequence unambiguously (pg. 8, lines 18-27).

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to have modified the method of Nyren et al with the blocked dNTP and 9N DNA polymerase (exo-) A485L/Y409V of Milton et al with the expected benefit of controlling the incorporation of nucleotide molecule complementary to the nucleotide in a target single stranded DNA to determine its sequence unambiguously as taught by Milton et al (pg. 8, lines 18-27).

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**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.

Regarding claims 2-4, Nyren et al teaches the steps of generating light as recited in said claims (Fig. 1, Middle panel)

Regarding claims 6 and 7, Milton et al teaches that moiety blocking the 3'-O- atom of the dNTP is an allyl moiety (pg. 5, lines 10-12).

Regarding claims 8-12, Nyren et al teaches that DNA is immobilized on a capillary solid substrate, using biotin- streptavidin interactions (Fig. 8).

Regarding claims 13-15, Milton et al teaches that the moiety blocking the 3'-O-atom of the dNTP is an allyl moiety and the single- stranded DNA is immobilized to a solid substrate (Fig. 5 and pg. 5, lines 10-12).

Claim 5 lack an inventive step under PCT Article 33(3) as being obvious over Nyren et al (USPN 6,210,891 issued April. 3, 2001) in view of Milton et al (WO 2004/018497 published Mar. 3, 2004) as applied to claim 1 above and further in view of Hanshaw et al (Tetrahedron Letters, 2004, 45, 8721-8724).

Regarding claim 5, Nyren et al and Milton et al are silent about determining whether pyrophosphate is generated by detecting dissociation of a coumarin-derived indicator from a complex between the indicator and a bis-Zn<sup>2+</sup>-dipicollylamine coordination compound. However, detecting dissociation of a coumarin-derived indicator from a complex between the indicator and a bis-Zn<sup>2+</sup>-dipicollylamine coordination compound was known in the art as taught by Hanshaw et al, who teaches a fluorogenic chemo sensing system wherein pyrophosphate are capable of displacing a fluorescent coumarin-derived indicator from a bis Zn<sup>2+</sup>-dipicollylamine coordination compound (Abstract). Hanshaw et al also teaches structure recited in claim 5 (Fig. 1, structure # 3). Hanshaw et al further teaches that dissociation of a coumarin-derived indicator from a complex between the indicator and a bis-Zn<sup>2+</sup>-dipicollylamine coordination compound is highly specific to pyrophosphate (Table 2).

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to have modified the method of Nyren et al and Milton et al with the pyrophosphate confirmatory method of Hanshaw et al with the expected benefit of having highly specific and an excellent quality control method for pyrophosphate detection as taught by Hanshaw et al (Table 2).

**CHAPTER II**  
**PCT TELEPHONE MEMORANDUM**  
**FOR**  
**LACK OF UNITY OF INVENTION**

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PCT No.: PCT/US06/24157

Examiner: NARAYAN K. BHAT

Attorney spoken to: Mr. Amos

Date of call: 20 August 2008

- Amount of payment approved:
- Deposit account number to be charged:
- Attorney elected to pay for ALL additional inventions
- Attorney elected to pay only for the additional inventions covered by
  - Group(s):  
-- encompassing --
  - Claim(s):
- Attorney elected NOT to pay for any additional inventions, therefore, only the first claimed invention Group 1, covered by Claim(s) 1-15 has been examined.
- Attorney was orally advised that there is no right to protest for any group not paid for.
- Attorney was orally advised that any protest must be filed no later than 1 Month from the mailing of the Opinion (Form PCT/IPEA/408) or the Final Report (Form PCT/IPEA/409).

**Time Limit For Filing A Protest:**

Applicant is hereby given 1 Month from the mailing date of this Opinion/Final Report in which to file a protest of the holding of lack of unity of invention. In accordance with PCT Rule 68.3, applicant may protest the holding of lack of unity only with respect to the group(s) paid for.

**Itemized Summary of Claim Groupings:**

**Detailed Reasons For Holding Lack of Unity of Invention:**  
Please See Continuation Sheet

**Note: A copy of this form must be attached to the Opinion/Final Report.**

**ATTACHMENT TO CHAPTER II PCT TELEPHONE MEMORANDUM  
FOR  
LACK OF UNITY OF INVENTION**

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**Detailed Reasons For Holding Lack of Unity of Invention:**

Attorney was contacted on August 20, 2008 and message was left to contact the examiner within 3 business days if attorney is interested to pay for the additional invention. Mr. Amos, Applicant's attorney contacted the Examiner on August 21, 2008, and confirmed that Applicant will not pay for the additional inventions.

*Note: A copy of this form must be attached to the Opinion/Final Report.*

**CHAPTER II**  
**PCT TELEPHONE MEMORANDUM**  
**FOR**  
**LACK OF UNITY OF INVENTION**

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PCT No.: PCT/US06/24157

Examiner: NARAYAN K. BHAT

Attorney spoken to: Mr. Amos

Date of call: 20 August 2008

- Amount of payment approved:
- Deposit account number to be charged:
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